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Carbon monoxide production in man was measured by both gas phase and by blood analysis. Both measurements correlated well with the simultaneous measurement of plasma bilirubin turnover, but the blood phase measurement is preferred since it is subject to less error. Correspondence of endogenous CO production and plasma bilirubin turnover indicate that heme destribtion is the major source of endogenous carbon monoxide in man.

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Chapter 11

COMPARISON OF PLASMA BILIRUBIN TURNOVER IN MAN WITH CARBON MONOXIDE PRODUCTION ESTIMATED SIMULTANEOUSLY BY BLOOD AND GAS MEASUREMENTS [1]

F. Lee Rodkey and Paul D. Berk [2]

Quantitative measurement of endogenous carbon monoxide (CO) production in man was first satisfactorily done by Coburn et al. (1963). It has now been established that the *in vivo* breakdown of heme results in equimolar production of CO and bilirubin (Landaw et al., 1970). Similar conversion of heme to CO and bilirubin was demonstrated in tissue preparations containing liver microsomes *in vitro* (Tenhunen et al., 1968). Procedures were devised to estimate the daily plasma bilirubin turnover (BRT) in man (Berk et al., 1969). Simultaneous estimations of plasma BRT and CO production in man have shown a high degree of correlation ($r=0.99$); however, the CO production averaged 114 percent of BRT for normal subjects and for subjects with increased heme turnover due to decreased red cell survival (Berk et al., 1974). This difference is partially attributed to the possibility that a small part of heme breakdown in the liver may lead to direct bilirubin excretion in the bile without passing through the plasma. Measurements of BRT would thus be expected to underestimate slightly total bilirubin production.

Procedures for measurement of CO production involve placing the subject in a closed rebreathing system to prevent CO excretion. The rate of change of CO in the total system is estimated from observed changes in blood carboxyhemoglobin saturation (COHb) (Coburn et al., 1963; Berk et al., 1974; Bensinger et al., 1971; Lynch and Moede, 1972) or from changes of CO concentration in the gas phase (Logue et al., 1971). Theoretically both blood and gas-phase analyses should lead to similar CO-production rates if sufficiently accurate data are obtained. This paper presents data for CO production obtained by simultaneous measurement of blood and gas-phase changes. These data are compared with BRT measurements performed at the same time.

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[2] Opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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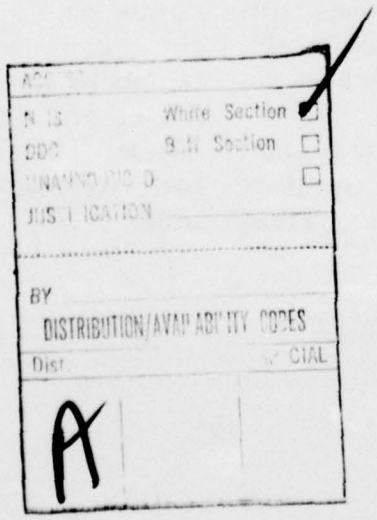

MATERIALS AND METHODS

Patients Studied

Simultaneous measurements of BRT and of CO production from blood and gas-phase data were performed in 12 individuals: 3 normal volunteers, 6 patients with congenital spherocytosis, 1 patient with sickle cell disease, 1 patient with paroxysmal nocturnal hemoglobinuria, and 1 patient with vinyl chloride exposure and subsequent hepatic fibrosis. BRT and blood-phase CO measurements, without gas phase data, were performed in an additional 37 individuals, as previously reported (Berk et al., 1974). Informed consent was obtained from each individual.

Plasma Bilirubin Turnover

Daily BRT was calculated from the plasma disappearance curve of radiolabeled unconjugated bilirubin (Berk et al., 1969). All studies were carried out for 30 hours and results expressed as μmoles/kg body weight per day.

Carbon Monoxide Production

The rate of CO production was measured in the closed rebreathing circuit shown in Figure 11–1. This procedure is essentially that of Coburn (Coburn et al., 1963) refined to provide an accurately controlled P_{O_2} in the closed system. Variation of P_{O_2} within the chamber of less than $\pm$ 2 mm Hg was achieved. Carbon dioxide of the inspired air in the closed system did not exceed 0.10 percent. A connection at the exit of the spirometer was used to remove samples of the gas phase for analysis

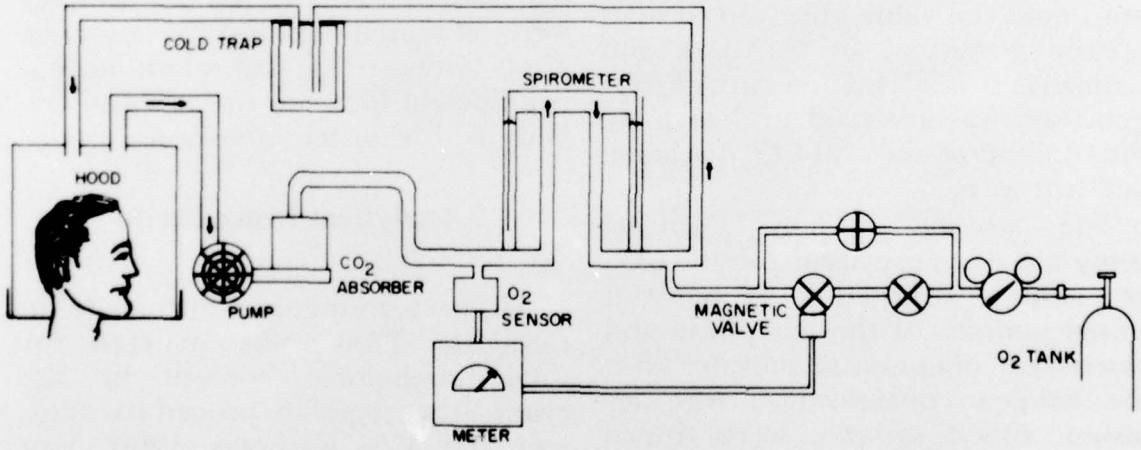

Figure 11–1. Schematic diagram of closed respiration system for measurement of carbon monoxide production. The confined gas, about 20 liters, was drawn from the hood by the pump, passed over the carbon dioxide absorber and oxygen sensor before entering the variable volume spirometer. The cold trap, packed in ice, served to cool the gas and remove water. Gas samples for analyses were drawn from a port on the spirometer.

as required and to add known amounts of CO for the dilution measurement. The entire volume of the closed system was measured (without the subject in the hood) and was 17.5 liters (actual volume) in addition to the calibrated variable volume of the spirometer. For purposes of calculation, the total gas volume was taken to be this measured volume plus the volume contained in the spirometer bell, ATPS,[3] and corrected to STPD.[3] The volume of gas displaced by the subject's head was assumed to be equivalent to the added lung volume.

Oxygen tension in the closed system was maintained near 150 mm Hg (F_IO_2 = 0.21).[4] Values of the "effective alveolar P_{O_2}" were calculated by the method of Riley et al. (1946) assuming arterial P_{CO_2} = 40 mm Hg and respiratory quotient (R.Q.) = 0.85. Use of 21 percent oxygen in the closed system provided estimated arterial P_{O_2} values from 95 to 100 mm, near the value observed in man breathing normal air by Clark and Lambertsen (1971). Each tank of oxygen used was analyzed to determine the contamination with CO, methane, and nitrogen.

The study was begun by the subject being placed in the hood and the oxygen content adjusted to 21 percent. Initial samples of the gas phase and blood were obtained 30 minutes after the oxygen concentration was adjusted. Blood samples were drawn from a peripheral vein by use of a small needle and a dry syringe. The blood was immediately placed in a small tube containing dry disodium ethylenediaminetetraacetic acid (EDTA), leaving the upper one fourth of the tube filled with air. The tube was stoppered and gently inverted to dissolve the anticoagulant and for mixing purposes before the analytic procedures. Additional samples of venous blood and of the gas phase were taken at 20-minute intervals over the next 2 hours. Usually six samples were used. The total-body CO-binding capacity (COBC) was then determined by 20 or 30 ml of CO (depending upon the subject's body wt) being added to the gas phase. The CO was added from an accurately calibrated double-stopcock glass tonometer filled with CO (99.9%, Matheson Co., East Rutherford, N.J.) at the known room temperature and atmospheric pressure. A gas-tight syringe was used to repeatedly flush the air from the closed system through the tonometer and back into the system. Venous blood samples were obtained 45 and 60 minutes after addition of CO when virtual equilibrium between the gas and the body had been reestablished.

Analytical Procedures

All measurements were made in duplicate. Blood was analyzed for total hemoglobin content by the cyanmethemoglobin procedure (Zijlstra and Van Kampen, 1960; Van Kampen and Zijlstra, 1961). A reaction time of 2 hours was used to insure complete conversion of COHb to cyanmethemoglobin (Rodkey, 1967). Blood CO content was determined by the method of Collision et al. (1968) as modified by Rodkey and Collision

[3] ATPS, gas volumes at ambient temperature and pressure water saturated; STPD, gas volumes reduced to 0°, 760 mm dry.

[4] F_ICO and F_IO_2, volume fraction of inspired gas which is carbon monoxide or oxygen.

(1970). Percent saturation of hemoglobin with carbon monoxide (COHb) was taken as 100 × blood CO content divided by the total hemoglobin COBC, i.e., 1.39 × g percent Hb. The gas phase was analyzed for CO by gas chromatography (Rodkey, 1970) and for oxygen and carbon dioxide by standard procedures.

Calculations

To calculate CO production from the blood data, the rate of COHb change was determined after the initial equilibration phase over about a 2-hour period. The method of least squares was used to establish the slope (ΔCOHb%) and intercept of this linear rate. A known STPD volume of CO (CO_D) was then added to the gas phase to determine the total COBC. The increment in COHb caused by CO_D was determined 45 and 60 minutes after the injection of CO. Corrections were made for the amount of CO_D remaining in the gas phase and for the endogenous change of COHb during the reequilibration period. CO production in ml/hr was calculated as ΔCOHb%/100 times COBC. Results were then converted to μmoles/kg body weight per day.

To calculate CO production from the gas data, the change of total CO in the gas phase (gas volume × fraction CO) was determined over the 2-hour period. The method of least squares was used to evaluate the slope (ΔCO_{gas}) expressed in ml/hr. Following reequilibration after addition of the known amount of CO (CO_D), the increment of total CO in the gas phase (ΔCO_D) in ml was calculated, taking into account the amount added to the gas phase from endogenous CO production during the reequilibration period. The CO production was then calculated from the equation

$$\text{CO production (ml/hr)} = \frac{CO_D \times \Delta CO_{gas}}{\Delta CO_D} \quad [1]$$

Results were then expressed as μmoles/kg body weight per day.

Total COBC was calculated from the gas data by use of the Haldane equation to obtain the change in COHb saturation caused by CO_D. The Haldane equation expresses the equilibrium relation

$$\frac{COHb}{O_2Hb} \times \frac{P_{O_2}}{P_{CO}} = K \quad [2]$$

where COHb and O_2Hb are the fractional saturations of hemoglobin with CO and O_2, respectively, and K is the relative affinity constant of hemoglobin for CO and O_2 (Rodkey et al., 1969). The equilibrium values, P_{CO} and P_{O_2}, are essentially those of arterial blood. In the closed rebreathing system alveolar, inspired, arterial, and equilibrium P_{CO} are identical, i.e., $P_aCO = (P_{Bar}-47)F_ICO$.[5] Equilibrium P_{O_2} is estimated as arterial P_aO_2 (Clark and Lambertsen, 1971; Riley et al., 1946; Rodkey et al., 1974), i.e., $P_aO_2 = (P_{Bar}-47)F_IO_2$-55 breathing air. The value of O_2Hb may be expressed as (1-COHb) when the inspired oxygen is 21 percent or greater. With these assumptions and approximations, COHb was calculated from the Haldane equation in the following form:

[5] P_{Bar}, barometric pressure; P_aCO and P_aO_2, partial pressure of carbon monoxide and oxygen in the arterial blood.

$$COHb = \frac{220\ P_aCO}{P_aO_2 + 220\ P_aCO} \quad [3]$$

The total COBC was then calculated as

$$COBC = \frac{100 \times (CO_D - \Delta CO_D)}{\Delta COHb} \quad [4]$$

where $(CO_D - \Delta CO_D)$ represents the CO absorbed by the subject and $\Delta COHb$ is the calculated change in COHb caused by the CO absorbed.

RESULTS

Variability of CO-Production Estimation From Blood Data

Variability of the CO-production estimation is a result of both biologic variation and analytic uncertainty in measurement of the COBC and of the rate of COHb change. Ten measurements of CO production and of COBC were made on a single normal subject over a 3-year period. The mean value for COBC was 11.9 ± 0.10 (SEM[6]) ml/kg, corresponding to a coefficient of variation (CV) of 2.5 percent. The corresponding mean value for CO production was 4.9 ± 0.28 μmoles/kg per day, CV = 18 percent, over the same period. This particular subject has a CO production lower than most normal individuals with a range of values from 3.0 to 6.2 μmoles/kg per day over the 3-year period. In a group of 6 male and 5 female normal volunteers (Blaschke et al., 1974) CO production was 8.9 ± 0.6 μmoles/kg per day with a range from 6.3 to 12.1 μmoles/kg per day corresponding to a 22 percent CV. Thus the day-to-day measurement of CO production for a given normal individual has a coefficient of variation similar to that observed among a group of normal individuals.

In view of the very small CV of COBC measured in a given subject (*vide infra*) and the very small CV's of the chemical methods employed, the analytic uncertainty of the CO-production estimation may best be expressed by the statistical error in the measured slope of COHb change. This error, expressed as the coefficient of variation of the calculated slope, has been estimated in 59 determinations on 32 individuals to be 8.0 ± 0.61 percent. The same error in slope is observed with male and female subjects with both normal and markedly elevated rates of CO production. A constant CV for wide changes in slope is probably due to the constant CV's for both total hemoglobin and blood CO content estimation (Van Kampen and Zijlstra, 1961; Collison et al., 1968). The data suggest that at least half of the observed uncertainty is due to biologic variation.

Equilibration of Added CO

Subjects placed on the closed rebreathing system equilibrate the gas phase to the equilibrium P_{CO} of the blood in less than 20 minutes. When CO is added to the gas phase, however, the reequilibration requires a longer period. This is illustrated in

[6] Standard error of the mean.

Table 11–1 where a subject was placed on the closed system and values of blood COHb and gas-phase CO were measured. Clearly, the fraction of the total CO in the gas phase approaches the theoretical value (1.54% for this study) within the first 20 minutes on the closed system. The same equilibrium distribution is achieved after addition of CO to the gas phase but at least 45 minutes are required. This represents the time for absorption from the gas and distribution through all CO binding compartments. For these reasons we have chosen to allow 45 minutes for reequilibration after addition of CO and have used data taken at both 45 and 60 minutes of reequilibration for duplicate estimates of COBC.

Comparison of CO Production Calculated From Blood and Gas Data

BRT and CO production were measured simultaneously. Values of CO production were calculated from the data obtained on the blood and from gas-phase analysis. The data are presented in Table 11–2 for subjects with and without evidence of hemolysis.

Clearly COBC can be estimated equally well from either blood or gas data. The ratio of COBC calculated from the gas data to that calculated from the blood data was 0.968 ± 0.01 for these 12 measurements. This value is not significantly different from 1.00 ($P > 0.2$) and the 95 percent confidence limits are 0.90–1.04.

There was generally good agreement between the 12 paired measurements of CO production in blood and gas phases ($r = 0.95$, $P << 0.01$). For these studies both gas- and blood-phase measurements of CO production correlated highly with BRT ($r = 0.97$ and $r = 0.96$, respectively). The ratio of CO production: BRT was 1.12 ± 0.14 for the 11 cases in

TABLE 11–1

COMPARISON OF GAS CO CONTENT AND THEORETICAL EQUILIBRIUM CO CONTENT: SUBJECT MALE, 58.6 kg, P_IO_2 = 172 mm Hg, P_{Bar} = 762 mm Hg, V_G = 18.68 L STPD, COBC = 914 ml*

Time on closed system (hr)	*COHb % sat.*	*CO in gas*		*Percent of CO in gas‡*
		Obs. (ppm)	*Calc.† (ppm)*	
0.33	0.54	4.16	4.02	1.55
1.00	0.57	4.36	4.24	1.54
1.97	0.66	4.85	4.93	1.48
2.00	CO added = 16.72 ml STPD			
2.27	2.43	55.5	18.5	4.46
2.52	2.49	21.5	18.9	1.73
2.77	2.56	20.2	19.5	1.59
3.02	2.54	20.5	19.4	1.58

*See text and text footnotes for explanation of abbreviations.
†Calculated by equation [3] with assumptions as given in text.
‡Calculated from observed V_G, CO in gas, COBC, and COHb.

TABLE 11–2

COMPARISON OF BILIRUBIN TURNOVER WITH CARBON MONOXIDE PRODUCTION*

Subject	*Sex*	*Diagnosis*	*51-Cr T½ days*	*BRT (μmoles/kg/day)*	*Blood Data* *COBC (ml/kg)*	*Blood Data* *CO production (μmoles/kg/day)*	*Gas Data* *COBC (ml/kg)*	*Gas Data* *CO production (μmoles/kg/day)*
D.H.	M	Normal volunteer	27.3	9.1	12.0	10.1	11.5	8.2
D.G.	M	Normal volunteer	....	....	15.8	9.9	15.6	7.3
P.R.	M	Normal volunteer	...	2.6	16.7	7.3	16.3	5.5
J.H.	F	Congenital spherocytosis post splenectomy	27.3	5.1	10.5	7.3	10.2	5.3
M.H.	M	Congenital spherocytosis	24.0	6.3	13.0	13.0	12.5	6.5
M.B.	F	Congenital spherocytosis	23.4	10.4	10.4	10.0	9.5	10.4
D.B.	F	Congenital spherocytosis	18.5	18.5	9.5	17.3	9.1	13.6
C.B.	M	Congenital spherocytosis	18.3	27.1	14.8	30.2	13.4	23.2
C.B.	M	Congenital spherocytosis post splenectomy	....	8.8	14.1	10.3	13.8	6.1
J.B.	M	Vinyl chloride liver damage	....	7.6	14.7	8.4	15.2	14.7
M.G.	M	Sickle cell disease	7.3	41.7	12.1	56.3	12.1	38.6
E.L.	F	Paroxsysmal nocturnal hemoglobinuria	....	29.1	9.3	28.3	9.1	26.7

*See text and text footnotes for explanation of abbreviations.

which both bilirubin and gas-phase data were available and was 1.18 ± 0.05 for the 48 studies from which both bilirubin and blood-phase CO data were available. In two patients in the present series, the ratio of CO production:BRT was greater than 2 for blood-phase measurements. This resulted from an extremely low and biologically unlikely value of BRT in subject P.R., and an extremely high value of CO production in subject M.H. We cannot explain either of these aberrant values; however, both are clearly inconsistent with ancillary observations. Although for the population studied, gas- and blood-phase methods gave equivalent data for CO production, the statistical uncertainty of any single study was much higher for the gas-phase data. The uncertainties of the two estimates of CO production are best expressed by the coefficients of variation in determination of the rate of change of ΔCOHb% in blood or of ΔCO_{gas} in the gas phase. The coefficient of variation in the slopes from these 12 studies was 8.7 ± 1.07 percent for the blood data and 26.9 ± 5.6 percent for the gas data. This significant difference, $P < 0.01$, is explained by the ex-

tremely small rate of change in gas CO concentration and the fact that over 98 percent of the CO stays in the subject and does not appear in the circulating gas volume. Another contributing factor is that small changes in gas volume from sampling or from leaks cannot be detected in the COHb estimation, but will be directly related to the calculated CO in the gas phase, and hence will change CO production as calculated from the gas data. The reliability of gas data increases for patients with elevated CO production. In the seven subjects with BRT < 10 a CV of 36.3 ± 7.8 was observed, while the five subjects with BRT > 10 had 13.7 ± 2.9, a value much closer to the coefficient of variation observed from blood data.

Effects of Constant Diet

Most CO-production estimates were made after the subject had eaten breakfast, but with no attempt to control or modify the diet. It is known that fasting (Bloomer et al., 1971) and severe caloric restriction (Lundh et al., 1972; Bensinger et al., 1973) increase serum bilirubin concentration and, to a smaller extent, CO production. A group of five normal volunteers was used to observe the variation in CO production when a constant diet was used. The diet was only controlled to the extent that adequate calories were provided and the subject consumed an identical menu on the day prior to and on the day of each of two or more measurements of CO production. Results given in Table 11–3 show that constancy of a diet containing adequate calories is not a major factor in eliminating variations in CO production. It appears that the observed upper and lower extremes of CO production in normal subjects, 6–11 μmoles/kg per day, may equally well be observed in a given individual on the constant diet (PR) or when the

TABLE 11–3

EFFECT OF DIETARY CONTROL ON REPLICATE ESTIMATION OF CARBON MONOXIDE PRODUCTION IN NORMAL VOLUNTEERS

Subject	*Sex*	*Dietary control*	*CO production (μmoles/kg/day)*	*COBC (ml/kg)*
VK	M	No	11.3	14.9
VK		Yes	6.6	15.0
VK		Yes	6.6	15.7
WK	M	No	12.5	16.8
WK		Yes	12.2	16.6
WK		Yes	12.9	16.8
RH	M	No	10.8	16.0
RH		Yes	11.8	15.8
PR	M	Yes	7.3	16.7
PR		Yes	6.6	16.3
PR		Yes	11.1	16.4
DG	M	Yes	9.9	15.8
DG		Yes	9.4	16.0
DG		Yes	11.1	15.4

diet is less controlled (VK). The uncertainty in estimation of the ΔCOHb%/hr for these subjects was never more than 10 percent and cannot explain the range of CO production observed in these two individuals.

DISCUSSION

This study confirms that measurements of CO production made in the gas phase are, on a statistical basis, highly similar to those obtained from the blood phase. The CO-production data from the two methods correlate highly with each other ($r = 0.95$) and both CO-production estimates correlate highly with measurements of BRT. Although the two CO methods are statistically similar, the gas-phase measurement is technically much more demanding and individual studies by this technique have a higher inherent statistical uncertainty especially at normal rates of CO production.

It is of interest that CO production exceeds BRT by approximately 12–18 percent whether measured in blood or gas phase. A small part of this discrepancy may result from the fact that BRT slightly underestimates total bilirubin production, as previously reported (Berk et al., 1974). Although it is widely accepted that bilirubin and CO are the products of heme catabolism in equimolar amounts and that neither arises from other sources, more recent studies show that CO may be produced biologically from nonheme sources such as dihalogenated methane (Kubic et al., 1974) and polyphenolic compounds including catecholamines (Miyahara and Takahaski, 1971). Furthermore, other studies (Engel et al., 1972) raise the possibility that a small fraction of the CO in expired air may arise from bacterial metabolism in the gastrointestinal tract. Despite these potential sources of "excess CO," the ratio of CO production:BRT observed in our studies suggests that in man the extent of extraneous CO production by such processes must be small or negligible.

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